Biomaterials 64 (2015) 21–32

Contents lists available at ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

Effects of cell–cell contact and oxygen tension on chondrogenic differentiation of stem cells

Bin Cao, Zhenhua Li, Rong Peng, Jiandong Ding*

State Key Laboratory of Molecular Engineering of Polymers, Department of Macromolecular Science, Advanced Materials Laboratory, Fudan University, Shanghai 200433, China

A R T I C L E I N F O

Article history:
Received 5 March 2015
Received in revised form
8 June 2015
Accepted 11 June 2015
Available online 15 June 2015

Keywords:
Chondrogenic differentiation
Mesenchymal stem cell
Cell–cell contact
Oxygen tension
Micropatterning
PEG hydrogel

A B S T R A C T

While cell condensation has been thought to enhance chondrogenesis, no direct evidence so far confirms that cell–cell contact itself increases chondrogenic differentiation of stem cells, since the change of cell–cell contact is usually coupled with those of other cell geometry cues and soluble factors in cell culture. The present study semi-quantitatively examined the effect of cell–cell contact in a decoupled way. We fabricated two-dimensional micropatterns with cell-adhesive peptide arginine-glycine-aspartate (RGD) microdomains on a nonfouling poly(ethylene glycol) (PEG) hydrogel. Mesenchymal stem cells (MSCs) were well localized on the microdomains for a long time. Based on our micropattern design, single MSCs or cell clusters with given cell numbers (1, 2, 3, 6 and 15) and a similar spreading area per cell were achieved on the same substrate, thus the interference of soluble factor difference from cell autocrine and that of cell spreading area were ruled out. After 9-day chondrogenic induction, collagen II was stained to characterize the chondrogenic induction results; the mRNA expression levels of SOX9, collagen II, aggrecan, HIF-1α and collagen I were also detected. The statistics confirmed unambiguously that the extent of the chondrogenic differentiation increased with cell–cell contact, and even a linear relation between differentiation extent and contact extent was established within the examined range. The cell–cell contact effect worked under both hypoxia (5% O_2) and normoxia (21% O_2) conditions, and the hypoxia condition promoted the chondrogenic induction of MSCs on adhesive microdomains more efficiently than the normoxia condition under the same cell–cell contact extents.

© 2015 Elsevier Ltd. All rights reserved.

1. Introduction

Cell-based cartilage tissue engineering is a promising approach for cartilage repair since impaired cartilage is difficult to self-heal due to the nonvascularized and noninnervated nature of any cartilage. The most important pertinent seeding cells used are chondrocytes and mesenchymal stem cells (MSCs). Previous studies on MSC-based cartilage tissue engineering focused on the optimization of chondrogenic induction protocols and the combination of MSCs with different scaffolds and stimulations to improve repair outcomes [1–5]. Among the key factors that facilitate successful restoration of cartilage lesion, cell condensation is of special importance, which is also a natural progress during cartilage formation *in vivo*. This process increases the extent of cell–cell contact, which might be beneficial for chondrogenesis as thought by many researchers [6–9].

However, once the cell–cell contact is altered by cell condensation or an increase of cell density, other cell geometry cues such as cell spreading sizes might also be altered, especially in the case of a two-dimensional (2D) culture. Meanwhile, the concentration of the soluble factors might also be changed by autocrine of the cultured cells with cell condensation. Therefore, decoupling of the cell–cell contact effect from those interference factors is essential in order to conclude the cell–cell contact effect solely on chondrogenic induction of stem cells. A deterministic experiment is thus strongly called for.

In the present study, we investigated the effect of cell–cell contact on the chondrogenic induction extent *in vitro* in a decoupled way, as schematically presented in Fig. 1A. A series of adhesive microdomains on a persistently nonfouling background were generated for the adhesion of single cells or cell clusters with given cell numbers, where the interference of different soluble factors could be ruled out. We also expected to fix other cell geometry cues such as cell sizes on microdomains. The prerequisite is localization of cell clusters with given cell numbers on cell-adhesive microdomains on the same substrate.

* Corresponding author.
E-mail address: jdding1@fudan.edu.cn (J. Ding).

http://dx.doi.org/10.1016/j.biomaterials.2015.06.018
0142-9612/© 2015 Elsevier Ltd. All rights reserved.

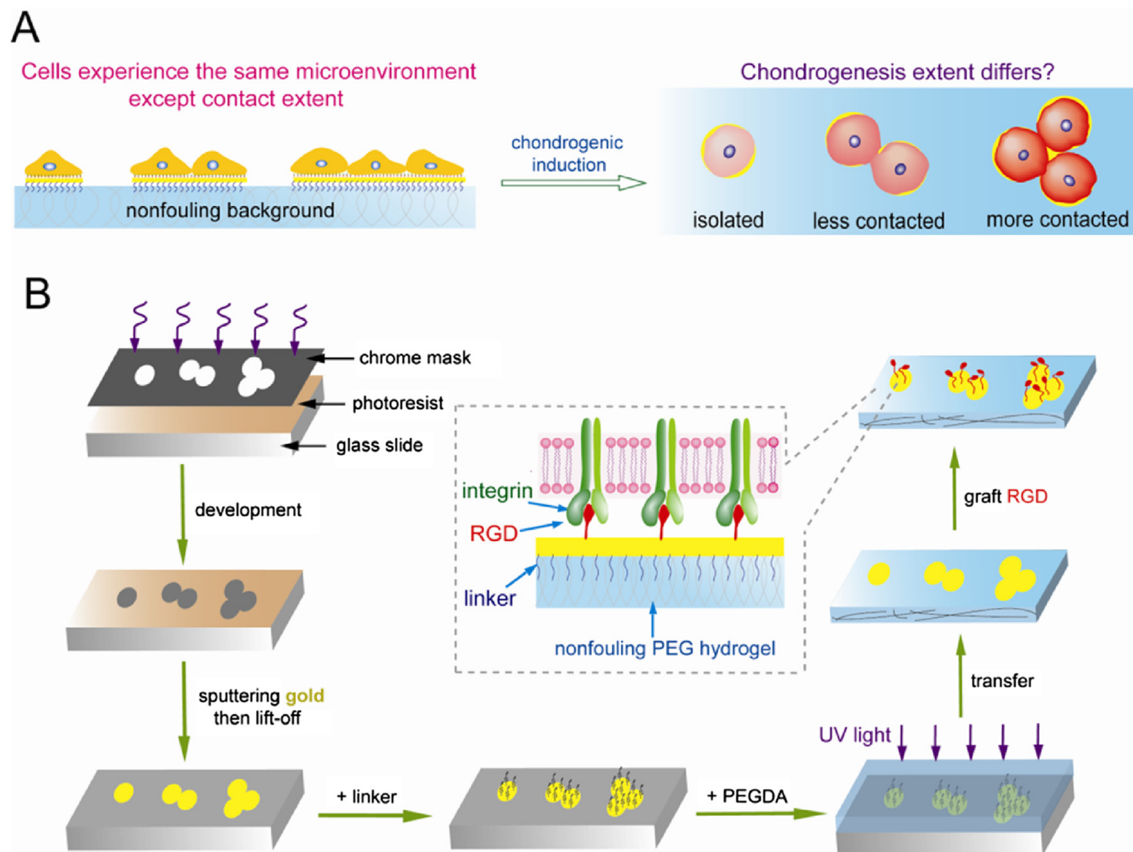

Fig. 1. A) Schematic presentation of the basic idea to examine whether cell–cell contact influences the chondrogenic induction. Stem cells on the microdomains of different microisland numbers experience the same microenvironment except contact extent. B) Fabrication flow chart of surface micropatterning with cell-adhesive microdomains of different microisland numbers on a persistent nonfouling PEG background. MSCs will be seeded on the micropattern and cultured in the chondrogenic medium for 9 days under hypoxia (5% O_2) and normoxia (21% O_2) conditions. The effects of cell–cell contact and oxygen tension on chondrogenic differentiation will be examined.

Various micropatterning and nanopatterning techniques for cell studies have been developed to explore the interaction between cells and biomaterials [10–15]. In this study, we employed an arginine-glycine-aspirate aspartate (RGD) peptide micropatterning technique on poly(ethylene glycol) (PEG) hydrogels that we previously reported [16–19]. The overall preparation process is schematically described in Fig. 1B. Briefly, the micropatterning technique includes three steps including (1) lift-off photolithography to generate a micropattern of gold on glass, (2) micropattern transferring from glass surface to the surface of a nonfouling poly(ethylene glycol) (PEG) hydrogel, and (3) formation of a self-assembly monolayer of cell-adhesive RGD peptide on the gold microislands, which can enhance cell adhesion [20,21]. The above three steps enable the successful acquisition of RGD microdomains on the persistently nonfouling PEG hydrogel. On these micropatterns, cells can be located within the microdomains for a long time.

In this study, we carried out the chondrogenic induction of MSCs on the micropatterned surfaces for 9 days in a chondrogenic medium. The micropatterns were designed to contain microdomains with microisland numbers 1, 2, 3, 6, and 15. Each microisland had the same area, which probably locates only one cell. The extent of cell–cell contact was thus well controlled by numbers of cells adhering on those microdomains that correspond with microisland numbers. Furthermore, the cell numbers on each microdomain will not change during induction, as adhesive areas are limited and a proliferation inhibitor aphidicolin will be introduced. Hence, the contact effect on the chondrogenic extent can be examined without the disturbance of other environment factors. While it has been recognized for a long time that cell–cell contact might enhance chondrogenesis, the present study will, based upon the unique micropatterning technique to control cell–cell contacts, set up the first semi-quantitative relation between *in vitro* chondrogenic induction extents and cell–cell contact extents.

In addition, low oxygen tension has been reported to be beneficial for the chondrogenesis of MSCs [22–28]. One possible reason is that MSCs proliferate faster in the hypoxia condition than that in the normoxia condition [29,30]. However, since faster proliferation in the hypoxia condition leads to higher cell–cell contact extents, it remains unknown whether the increased chondrogenesis is caused directly by hypoxia or indirectly by higher cell–cell contact extent derived from higher cell proliferation. One strategy to answer this argument seems to be from examination of chondrogenic induction of MSCs in parallel experiments of different oxygen conditions but under the same cell–cell contact (say, with the same initial cell density on tissue culture plates) with addition of a proliferation inhibitor such as aphidicolin. However, according to our trial, it is impossible to keep the same global cell density in culture under the two oxygen conditions with a fixed inhibitor concentration; on the other hand, if one increased the aphidicolin concentration in the hypoxia condition, the inhibitor concentration became another interference factor, which makes the comparison between the "direct" effect of the hypoxia condition versus the normoxia condition still not conclusive. Taking advantage of our micropatterning

Download English Version:

https://daneshyari.com/en/article/5625

Download Persian Version:

https://daneshyari.com/article/5625

Daneshyari.com